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SYNTHESIS OF 6-SUBSTITUTED-2,4-DIAMINO-5,6,7,8-TETRAHYDROPYRIMI--ETC(U)
JUL 81 T J DELIA, S M SAMI, D D KIRT DAMD17-78-C-8004
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REPORT NUMBER 7
(Revised)

Synthesis of 6-Substituted-2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidines

Final Report

by

Thomas J. Delia, Salah M. Sami and Diane D. Kirt

(Reporting Period: 1 October 1977 - 31 March 1981)

JULY 1981

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701

Contract No. DAMD-17-78-C-8004
Central Michigan University
Mt. Pleasant, MI 48859

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD A104571
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Synthesis of 6-Substituted-2,4-Diamino-5,6,7,8-Tetrahydropyrimido (4-5-d) Pyrimidines Revised.	5. TYPE OF REPORT & PERIOD COVERED Final Report. 1 Oct 77-31 Mar 81
	6. PERFORMING ORG. REPORT NUMBER TJD-1981-7
7. AUTHOR(s) Thomas J. Delia, Salah M. Sami, and Diane D. Kirt	8. CONTRACT OR GRANT NUMBER(s) DAMD-17-78-C-8004
9. PERFORMING ORGANIZATION NAME AND ADDRESS Central Michigan University Mt. Pleasant, Michigan 48859	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A 3M162770A803 00.039.
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Fort Detrick, Frederick, MD 21701	12. REPORT DATE Jul 1981
	13. NUMBER OF PAGES 29
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Tetrahydropyrimido (4,5-d) pyrimidines
Mannich reaction
Arylamines
Benzylamines
Benzylhydroxylamines
Phenylethylamines
Antimalarial
Antitrypanosomal

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A series of sixteen target compounds involving primarily methylene ethylene and methyleneoxy spacers between the heterocyclic portion and halogenated phenyl portion were prepared and submitted for screening. The chemistry and biological screening results are described. None of the compounds exhibited useful chemotherapeutic activity.

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I. Summary

A series of 6-substituted-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidines were prepared from 2,4,6-triaminopyrimidine and the corresponding omines in the presence of formaldehyde. This modified Mannich reaction produced the target compounds in a one pot yield of better than fifty percent in each case.

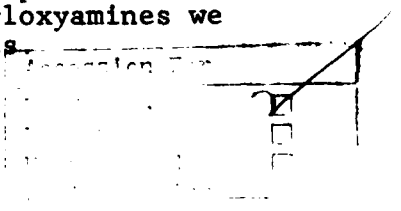
The rationale for the synthesis of these compounds was based on analogy to previously reported structures of dihydrofolate reductase inhibitors which possessed antimalarial activity. None of the compounds reported herein were found to have antimalarial activity.

In view of the negative results obtained so far with a series of 2,4-diamino-6-substituted-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidines we are drawn to a number of conclusions.

Since one of the rationales for proposing this series of compounds was the potential antifolate activity it is surprising to find little antimalarial activity which would presume this enzyme inhibition. On the surface there appears to be very little difference in the ring system proposed, namely the tetrahydropyrimido[4,5,-d] pyrimidine and the tetrahydropteridine ring of folic acid. An examination of molecular models does indicate that the N-substituent would necessarily have to lie out of coplanarity with the ring system albeit still equatorial. This is true for both ring systems.

There is another factor involving spatial relationships. The compounds tested so far possess from one to four atoms intervening between the chlorinated phenyl ring thought to be necessary and the reduced heterocyclic ring system. There does not appear to be any informative pattern in the biological response. A majority of members of each class exhibit some toxicity at high dose levels without any indication of useful activity at lower levels. Thus we have to conclude that an essential feature of antimalarial activity is missing.

Based on the foregoing biological results and conclusions we do not see continuation of further members of the original target compounds as cost effective. Because of extreme difficulties and time-consuming efforts in the preparation of various x-phenylpropylamines and x-phenylpropyloxyamines we have abandoned these remaining target compounds.



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FOREWARD

The principal investigator and all other research personnel involved in activities described herein are grateful to the U.S. Army Medical Command for its financial support through Contract No. DAMD-78-C-8004. The continuing advice and support of Drs. Thomas Sweeney, Richard Strube, Robert Pick and Hikmat Musallam of WRAIR is likewise acknowledged.

No chemical research devoted to the development of chemotherapeutic agents can be successful without considerable help from those experienced in the biological aspects of screening. The authors are most appreciative of the efforts of Dr. Arba Ager of the University of Miami for the biological screening and of Dr. D.E. Davidson of WRAIR for the interpretation of the biological data.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals Resources, National Research Council (DHEW Publication No. (NIH)78-23, Revised 1978).

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II. Introduction

A. Period of Contract Activity. Initial approval of this contract was received in early October, 1977. However, it was not until March 1978 that work formally began in the laboratory. The delay was due to the difficulties associated with the date of award and final approval of the contract. These difficulties included a three month delay for university approval and the subsequent hiring of the required personnel to conduct the research. Other extensions were sought throughout the contract period for a variety of reasons. Thus, even though only two years of official support was provided the contract covered the period of October 1977 through March 1981.

B. Contract Personnel. During the course of this contract three individuals were supported in whole or in part by contract funds. The major support went to Dr. Salah M. Sami who was the post-doctoral research associate responsible for most of the work during the period of March 1978 through February 1980. During this period and subsequent to Dr. Sami's departure Ms. Diane D. Kirt played a key role in the synthesis of some of the target molecules as well as developing the methodology for key intermediates in the project plan.

These people were aided by two undergraduates at different times. Ms. Theresa Higgins provided able support for Dr. Sami during the spring and summer of 1978 and Mr. Kevin Perry aided Ms. Kirt in the winter of 1980. Both of these individuals were supported by research funds provided by Central Michigan University.

III. Project Rationale

A. Chemical Rationale. A survey of the literature of antimalarial agents led us to consider two main areas. These were the antifolates, as represented by the quinazoline class of compounds and the dihydro-s-triazines. A brief description of some of the salient features necessary to outline our proposed plan of research follows. The references are not meant to be complete, but rather representative.

1. Antifolates. The discovery of the antiprotozoan activity of 2,4-diaminoquinazolines led to the syntheses of a wide variety of compounds of this class (1). Such compounds may be illustrated by the structure shown in Figure 1. Hynes and Ashton (2) have summarized the structure activity patterns which have evolved for compounds of this type:

a. for pyrimidines and pteridines the 2,4-diamino configuration affords optimal activity.

b. an aromatic substituent at position 6 is usually necessary for potent activity.

c. the introduction of a small hydrophobic group such as -Cl or $-CH_3$ at position 5 sometimes enhances activity.

d. a variety of species may be employed in bridging the aryl group to the quinazoline nucleus (for example, Y in Figure 1). These authors inserted methylene, oxymethylene, and thiomethylene units in their studies.

Substituents on the aromatic ring which have shown significant activity are the 4-Cl (3), $3,4-Cl_2$ (3,4) and CF_3 (5). Spacers such as the Y = $-CH_2-$ (6) were also reported to be effective. Elslager et al. (7) reported that 2,4-diamino-6-[3,4-dichlorobenzyl] amino quinazoline and other 2,4-diamino-6-{[aralkyl and (heterocyclic) methyl] amino} quinazoline antifolates display strong antimalarial effects.

Finally, reports of significant activity associated with tetrahydroquinazoline derivatives were important considerations (8,9). The specific interest in this feature is derived from the fact that one example, 2,4-diamino-5,6,7,8-tetrahydroquinazoline is three times as effective as its fully aromatic analog (4).

2. Dihydro-S-Triazines. The metabolite cycloguanil [4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s-triazine] was among the first of this class of chemical compounds to demonstrate activity against plasmodia (10).

Subsequently, a variety of substituted 1,2-dihydro-s-triazines have been prepared as antimalarial agents. These have been reported in both the journal literature and the patent literature. Such compounds can be represented by the structure shown in Figure 2 in which R_1 and R_2 are typically alkyl groups of various sizes with the most prevalent examples containing the dimethyl moieties. However, R_3 has shown more variation in the search for active compounds. For example, Laing (11) has shown that the derivative in which $R_1 = R_2$ = methyl and R_3 = 3,4-dichlorobenzyloxy has potent activity against P. berghei, including strains of the parasite resistant to chloroquine, dapsone, sulfonamide, and pyrimethamine, respectively. Studies by Lee (12) of this compound in

Figure 1

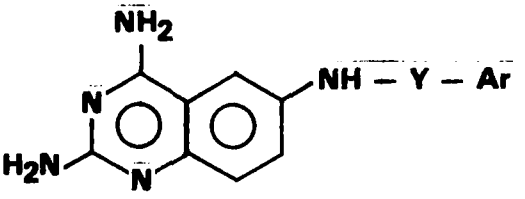
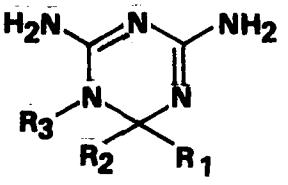


Figure 2



beagles suggests that toxic doses inhibit the reduction of folic acid to a tetrahydro derivative and thus interferes with the transfer and utilization of a single carbon moiety which is essential for the plasmodial metabolic pathway. Other derivatives of the benzyloxy analog include the mono- and tri-chloro moieties.

Expansion of this approach has led to a variety of spacers between the aromatic moiety and the heterocyclic ring. Mamalis (13) has synthesized compounds in which R_3 = 3,4-dichlorophenylpropyloxy; and alternatively where R_3 = 2,4,5-trichlorophenoxypropyl (14); and still another variation is provided by the example where R_3 = 2,4,5-trichlorophenoxypropyloxy (15).

A related example of this type of side chain is found in a series of N-(ω-aminoalkoxy)-phthalimides (16). Such compounds were considered likely candidates because of their structural resemblances to febrifugine and isofebrifugine.

B. Biological Rationale. Scheme 1, reproduced from Thompson and Werbel (17), serves both as a summary of some of the previous discussion and the focal point for the following suggestions. In it the major classes of inhibiting agents are seen at their sites of action.

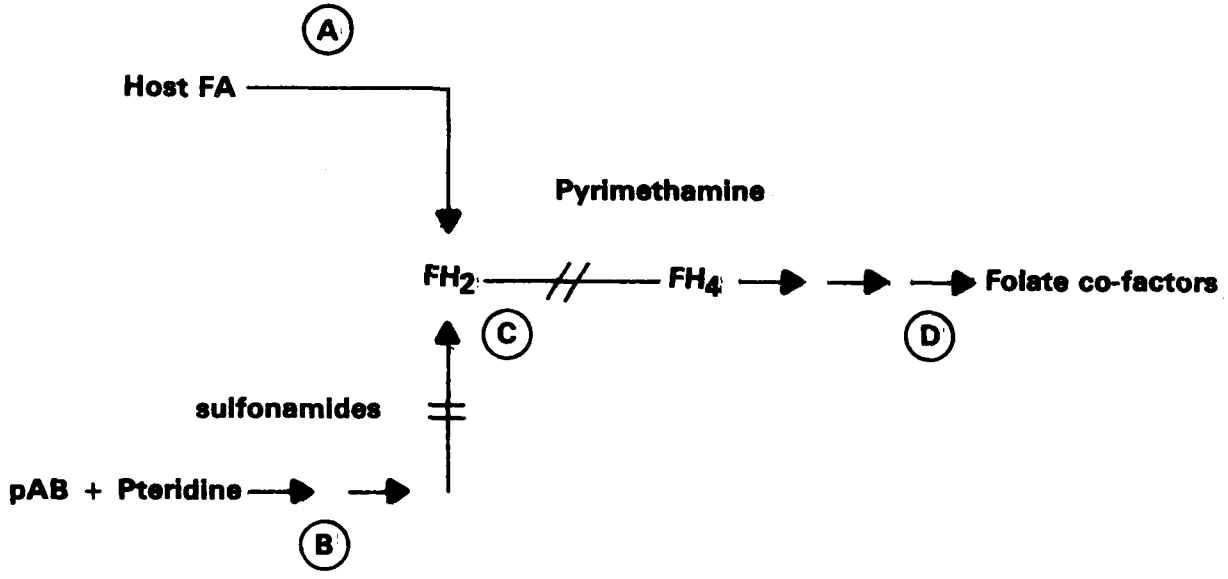
Thus, the mechanisms of action of the sulfonamides is by inhibition of dihydropteroate synthetase and that of the antifolates is by tightly binding to the plasmodial dihydrofolate reductase.

Bungener and Nielsen (18) found that tritiated thymidine and uridine were not incorporated into the nucleic acid of P. berghei and P. vinckei. This supports the suggestion by Ferone (19) that plasmodia need to synthesize the folate-containing cofactors de novo, and that they are unable to utilize preformed folates.

All of this evidence places increased emphasis on the synthesis of the folate cofactors since precursors to these cofactors are essential for plasmodia reproduction. Thus, classes of compounds possessing antimalarial activity which could interfere with the folic acid-dependent methylation of deoxyuridylate to form thymidylate are of interest in addition to the other sites for interference. The combination of two drugs that inhibit parasite growth by acting at two different sites in the folic acid metabolic pathway is a valued goal. In this regard, it is noteworthy that folate metabolism in P. lophurae is restricted to the thymidylate synthesis cycle (20).

In this cycle, inhibition of dihydrofolic acid reductase

SCHEME 1


A
Host FA
Pyrimethamine
FH2
FH4
Folate co-factors
C
D
sulfonamides
pAB + Pteridine
B

by antifolates such as pyrimethamine blocks the regeneration of the tetrahydrofolic acid, resulting in the cessation of thymidylate synthesis, and thereby stopping DNA synthesis and reproduction of the parasite (21).

Further interest in this approach is provoked by the observation that thymidylate synthetase in mouse erthrocytes infected with P. berghei increases markedly during cellular parasitization (22).

Finally, it has recently been reported that in plasmodia thymidylate is synthesized, apparently, only by thymidylate synthetase (23).

All of the agents thus far have focused on the inhibition of dihydrofolic reductase specifically. Since dihydrofolic reductase is needed to convert dihydrofolic acid to tetrahydrofolic acid, and via folinic acid to 5,10-methylene tetrahydrofolic acid which is the methylating agent for the synthesis of thymine, this leads to inhibition of thymidylate synthetase. It is possible that a competitive inhibition of the 5,10-methylene tetrahydrofolic acid would be a reasonable alternative (24).

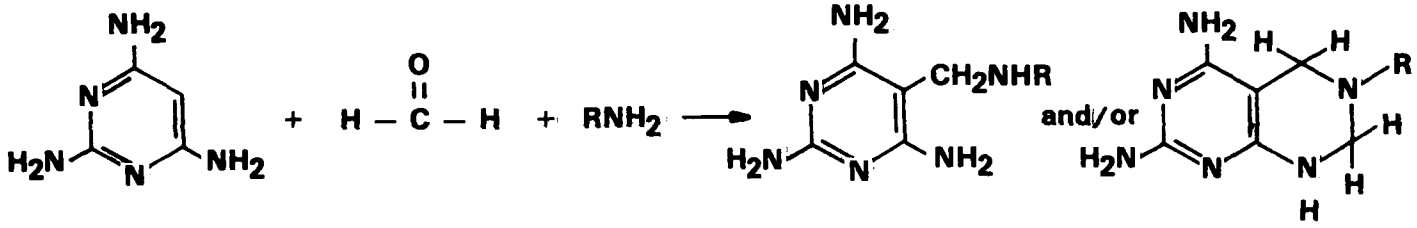
It is with this possibility in mind that we proposed the tetrahydropyrimidopyrimidines as a viable class of compounds for screening as antimalarials.

In proposing the 5,6,7,8-tetrahydropyrimido (4,5-d) pyrimidines, we included some of the most desirable features of each of the active classes previously discussed. Thus we proposed only the synthesis of 2,4-diamino derivatives with variable substituents in the 5- and 6- positions. The 6- substituents all possess an aromatic moiety which bears either Cl of CF_3 in a variety of positions on the ring. The aromatic ring was intended to be separated from the heterocyclic ring by zero to five atoms in length. Finally, we proposed only tetrahydro derviatives in which the ring bearing the aromatic substituent is reduced.

C. Synthetic Plan. In our laboratory we have been investigating the Mannich reaction as applied to pyrimidines (25). During the course of these studies we carried out the reaction shown in Scheme 2.

Compound 4 was expected as the major component. But where R = butyl or p-MeO-phenyl the resulting products (5) were tetrahydropyrimidopyrimidines. It is this double methylene insertion reaction that we proposed as the basis for a synthetic program designed to provide a series of compounds having the tetrahydropyrimido-pyrimidine ring and a series of R groups (5) shown to be

SCHEME 2



1 2 3 4 5

of interest as antimalarial moieties. Thus, the series of compounds shown in Figure 3 were proposed.

In the event that such derivatives were not readily obtained in one step, cyclization of the Mannich bases (e.g. 2) could proceed readily in a second step in accordance with the reaction of Harmon (26) as shown in Scheme 3. The synthesis of 3 described above requires the preparation of amines ($R\bar{N}H_2$) described in Figure 4 which are readily available, either commercially or through well described procedures reported in the literature.

IV. Chemistry

A. Approach to the Problem.

The proposed chemical synthesis of the title compounds has been described in Scheme 2. The 6-NH_2 group adjacent to the initial site of attack is a necessary component of this reaction. We have previously investigated other pyrimidines in which the 2- and 5- substituents were varied but did not always include the 6-NH_2 group and found that the reaction did not proceed to the corresponding 5,6,7,8-tetrahydro-pyrimido[4,5-d] pyrimidines.

Before we began this work we did not expect any difficulties with the various amines proposed. However, it is now clear that each series of amines has a different feature which ultimately resulted in poor to excellent results.

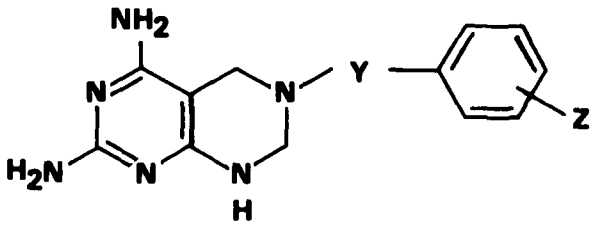
We have put emphasis on the synthesis of compounds that would bear aryl, aralkyl, O-aryl and O-aralkyl substituents in position 6. Each of these contains one or more halogen atoms (Cl or F) attached either directly or indirectly to the aromatic ring. In this report we describe the attempts and results achieved towards the synthesis of these compounds.

B. Discussion of Results

1. Reactions with Phenylethylamine and its Derivatives (3e-j).

The reaction of 1 with amines under the Mannich conditions as well as the yield of the reaction depends largely on the structure of the amine used, molar ratio of the reactants, temperature, pH of the media, solvent, reaction time, and other conditions affecting the course of the reaction. In our preliminary experiments we investigated the reaction using a variety of combinations of these parameters with the commercially available phenylethylamine as a model. Experiments were performed over a temperature range of 0-80°C, a variety of molar ratios

Figure 3



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Y = — O — $(CH_2)_{0-3}$ — ; Z = —Cl, di-Cl, CF_3

Y = — O — $(CH_2)_{0-3}$— O — ; Z = — Cl, di-Cl, CF_3

Y = —$(CH_2)_{0-3}$— ; Z = —Cl, di-Cl, CF_3

SCHEME 3

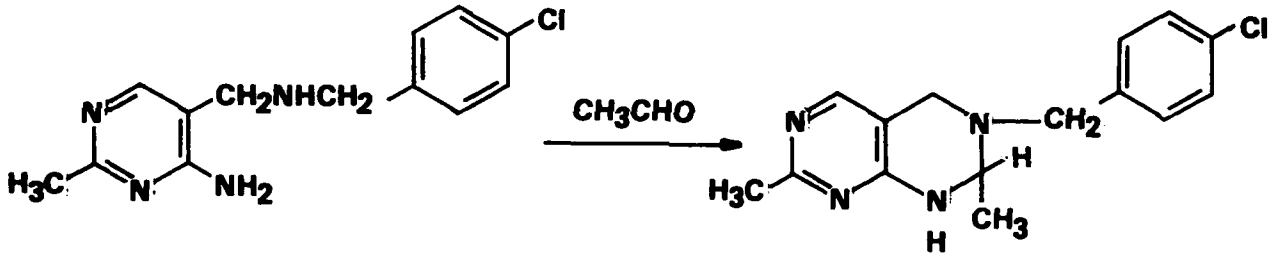
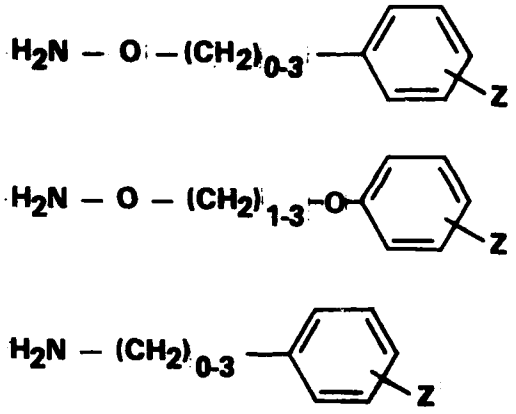


Figure 4



Where Z = monochloro, dichloro, or trifluoromethyl

of 1:3:2 including 1:1:1, 1:1:2, 1:2:2 and using water, aqueous ethanol or absolute ethanol as solvent. 1 and the amine were added into the reaction as either the free bases or in the form of their hydrochloride salts. In some cases the reaction was carried out with the free bases in the presence of a few drops of conc HCl (pH≈4). In some experiments the Schiff base of the amine or its hydrochloride was first prepared in situ, then allowed to react with 1. In all experiments intermediate 4 could not be detected or isolated. The main reaction products in the early studies were either unidentifiable solids (the majority of which were insoluble in most common solvents but dissolve in some organic acids like formic, acetic and lactic acids) or unreacted starting materials along with other minor components.

Six compounds in this series were finally prepared and characterized. The series included three monochlorophenyl derivatives (2-Cl, 3-Cl and 4-Cl), two dichlorophenyl derivatives (2,4-Cl_2 and 2,6-Cl_2) and one trifluoromethyl derivative (3-CF_3). These compounds were ultimately achieved in yields exceeding 60% by reaction of 2,4,6-triaminopyrimidine, formalin and the corresponding phenethylamines in a ratio of 1:2:2, with premixing of the latter two reagents. The reaction proceeds best in a basic medium.

The requisite phenethylamines were obtained commercially or prepared by hydride reduction of the corresponding benzyl cyanides. The physical and chemical data of all new compounds are reported in Table 1.

2. Reactions with Benzylamine Derivatives (3a-d). We undertook next the examination of the reaction with the benzylamine series. When 1 was allowed to react with 3a (3,4-dichlorobenzylamine hydrochloride) and polyoxymethylene in a molar ratio 1:1:1, respectively, with boiling ethanol as solvent, the main isolable products were a yellow insoluble solid, unreacted 1 and unreacted amine (3a). In boiling acetic acid with the same molar ratio of the reactants but using the benzylamine base instead of the salt the reaction led to the formation of insoluble yellow solid, unreacted amine and a very poor yield of the tetrahydropyrimidopyrimidine 5a. When the reaction with the hydrochloride salt of the amine was carried out in the same manner as in the first case but with a molar ratio 1:1:2 and without prior formation of the Schiff base we obtained after 24 h. refluxing a mixture of insoluble yellow solid and 5a in nearly equal amounts. In basic medium the reaction seemed

to favor tetrahydropyrimidopyrimidine formation. Thus, equimolar quantitites of 1 and the Schiff base of 3,4-dichlorobenzylamine (prepared from equimolar amounts of the amine and formalin) reacted within 24 h. refluxing in ethanol to give a mixture of 5a (≈30%) and 5-hydroxymethyl-2,4,6-triaminopyrimidine (6)(Figure 5)(≈50%). However, the best results were obtained when this experiment was repeated employing a molar ratio of 1 to the Schiff base of 1:2. In this case 1 reacted smoothly without the formation of side products to give nearly a quantitative yield of compound 5a. Compounds 5a-d as well as their hydrochloride salts were prepared successfully in a similar manner. Note should be made to the fact that in all these experiments we were unable to detect the existence of 4a-d among the products. The physical and chemical data of all new compounds in this series are reported in Table 1.

3. Reactions with O-Benzylhydroxylamine Derivatives (3k-n). The reaction of 2,4,6-triaminopyrimidine with O-benzylhydroxylamines and either formalin or polyoxymethylene under acid-catalyzed conditions provides moderate yields of the corresponding 6-benzyloxy-2,4-diamino-5,6,7,8-tetrahydropyrimido-[4,5-d] pyrimidines (5k-n). These products are also indicated in Table 1. It is not possible in this series to completely eliminate the formation of the bis pyrimidylmethane side product, 7 (Figure 6). This product and cleavage of the O-C bond leading to 6-hydroxy-2,4-diamino-5,6,7,8-tetrahydropyrimido-[4,5-d] pyrimidine (8) (Figure 7) accounted for the poorer yield of these benzyloxy compounds.

   The requisite O-benzylhydroxylamines were usually prepared from the corresponding benzyl halides and N-hydroxyphthalimide, followed by hydrazinolysis of the phthalimide intermediate.

   The physical and chemical data of the derivatives in this series are reported in Table 1.

4. Reactions with Anilines Derivatives. We have also investigated the Mannich reaction of 1 with aromatic amines, namely p-chloroaniline, o-chloroaniline and 2,4-dichloroaniline. These reactions are carried out under the same conditions that worked so well with the benzylamine series. Among these amines only p-chloroaniline reacted in the desired direction.

   We succeeded in isolating the product in poor yield (18%) from the reaction product which consisted mainly of unreacted amine and a large amount of an unidentifiable solid insoluble in most common solvents.

Figure 5

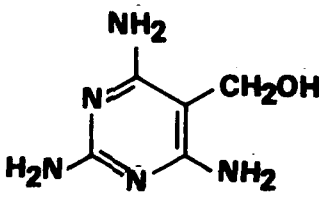


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Figure 6

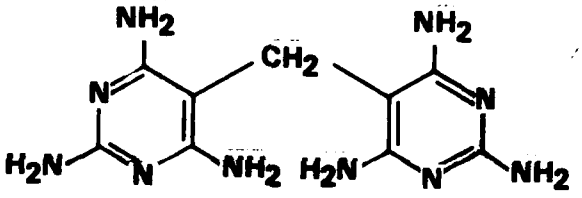

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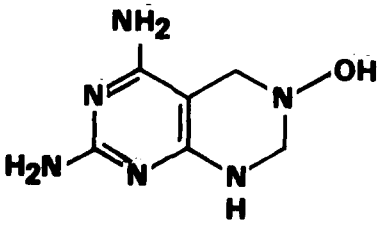
Figure 7



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However, we were not able to purify this material sufficiently for characterization. The other examples gave a large amount of unidentifiable yellow solid which was insoluble in all solvents tried together with unreacted amine while besides these two products, a compound thought to be 7 (Figure 6). In neither case could we detect even traces of the desired compounds. When the reaction was repeated with o-chloroaniline and 2,4-dichloroaniline under the same conditions mentioned above but using a molar ratio of 1:3:2 of 1:4:4 we obtained essentially the same results.

5. Reactions with Miscellaneous Amine Derivatives

   a. Miscellaneous Aralkoxy and Aryloxyalkyl amines. The reaction of 2,4,6-triaminopyrimidine with 2-(4-chlorophenyl)-ethoxyamine (3p) and formaldehyde was successfully employed to form 6-[2-(4-chlorophenyl)ethoxy]-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidine (5p) in 62% yield. A similar reaction using 2-(2,4-dichlorophenyloxy)-ethoxyamine (3o) was successful, although the yield was only 22%. In both cases significant quantities of the previously mentioned bis pyrimidyl methane, (7), were isolated.

   Other attempts utilizing either one or two oxygens in the space between the amine function and the aryl moiety have been made. On the whole considerable difficulty was encountered with either the synthesis of the requisite amine or the subsequent reaction with 2,4,6-triaminopyrimidine. These will not be detailed further since they were not pursued to a final conclusion.

   b. Hydroxylamine. The reaction between 2,4,6-triaminopyrimidine and hydroxylamine with formaldehyde was investigated. The product from this reaction, 6-hydroxy-2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidine, could be used as an intermediate in those series described above which proved difficult. These involved the N-O-C system which we found to be susceptible to cleavage at the O-C bond.

   The compound was ultimately isolated in 70% yield by allowing the mixture to remain at room temperature for one week. Satisfactory elemental analysis of this compound has been achieved. Biological screening, however, was not carried out with this molecule.

c. 2-Hydroxybenzylamine. The synthesis of 6-(2-hydroxybenzyl)2,4-diamino-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidine was attempted. This new target compound arose from discussions with Dr. Richard D. Strube in which the particular arrangement of the $N-CH_2-C=C-OH$ function was deemed a possible active variation of our hitherto unrewarding benzyl series. The 2-methoxy and 2-benzyloxy derivatives were utilized, in turn, to try to achieve this synthesis. Each of these compounds participated in the formation of the corresponding 6-substituted pyrimido[4,5-d] pyrimidine. However, either decomposition or very poor yields were associated with all attempts to remove the blocking groups. This approach was finally abandoned.

V. Experimental. Melting points are uncorrected. PMR Spectra were recorded in DMSO-d_6 on a Varian T-60 spectrometer with TMS as internal standard. Mass spectra were performed by The Walter Reed Army Institute of Research. All the benzylamines used were available commercially. The phenylethylamines used are known and were prepared by the $NaBH_4$-$CoCl_2$ reduction of the corresponding cyano compound (27). The benzylhydroxylamines are also known and were prepared according to a known general procedure (28).

General Method for the preparation of 2,4-diamino-6-aralkyl-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidines (compounds 5a-j). A mixture of two equivalents of the appropriate amine and two equivalents of formalin (37%) was shaken at room temperature without solvent for 15 minutes. The gummy material obtained was then treated with a solution of one equivalent of 2,4,6-triaminopyrimidine in ethanol. After refluxing for 24 h. the clear reaction mixture was evaporated under reduced pressure and the gummy residue solidified upon washing once with petroleum ether (63-74°) followed by ether several times. In some cases, after washing with petroleum ether, the gummy material was dissolved in the least amount of MeOH (cases e and g) or ether (case j) and the resulting solution was allowed to stand in the refrigerator for 24 h. to give a white crystalline material. Purification of the products was achieved by several crystallizations from the proper solvent or by silica gel column chromatography. Solvents used were $CHCl_3$-MeOH (9:1), (8:2) or (7:3) followed by crystallization. When a mixture of 3,4-dichlorobenzylamine (0.88 g, 5 mmole) and 37% formalin (0.41 g, 5 mmole) was treated with a solution of 2,4,6-triaminopyrimidine (0.625 g, 5 mmole) in 30 ml Ethanol and the resulting solution heated under reflux for about 2 h. a white precipitate formed. After continued refluxing for 24 h. the reaction mixture was cooled to room temperature and the white precipitate

(0.35 g) was filtered and recrystallized from dimethylsulfoxide or water (sparingly soluble). This material is 5,5-methylene bis-2,4,6-triaminopyrimidine, mp>360°. The PMR spectrum indicated absorptions at δ 3.34 (2H,S,-CH_2), δ 5.3 (2H,S,NH_2 at position 2), δ 5.6 (4H,S,NH_2 at positions 4 and 6). The mass spectrum show major ions at m/e 262(M^+), m/e 138, 125 and 110. The ethanol filtrate upon evaporation and the residue treated as described gave 0.5 g of 5a.

General Method for the preparation of 2,4-diamino-6-aralkoxy-5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidines (compounds 5k-p).

A mixture of two equivalents of the appropriate hydroxylamine hydrochloride derivative and two equivalents of polyoxymethylene was refluxed in 95% ethanol (14) while stirring for 5 h. The oxime solution thus obtained was treated with a solution of one equivalent of 2,4,6-triaminopyrimidine in ethanol. After refluxing and stirring for 2 h. a white precipitate separated from the reaction medium. Refluxing and stirring was continued for 26 h. The cold (room temperature) reaction mixture was made alkaline by stirring with sodium hydroxide pellets at room temperature and the insoluble precipitate was filtered. This included the methylene bis-2,4,6-triaminopyrimidine. The ethanol filterate was evaporated under reduced pressure and the residue obtained was washed with ether then purified either by several crystallizations (case 1) or by chromatography on a silica gel column (solvent $CHCl_3$-MeOH, 9:1) followed by crystallization from an appropriate solvent to give the title compounds.

VI. Screening Data. Sixteen target compounds have been submitted for screening. These compounds and their corresponding code numbers and bottle numbers are shown in Table 2.

A. Antimalarial Screening. Fifteen of the sixteen compounds submitted (except 5p) have been screened in the standard rodent procedure (29). None of the target compounds exhibited sufficient activity to warrant continuation of the series. In the case of compound 6n a certain degree of low level activity was noted. However, this could not be substantiated upon rescreening. In all, twelve compounds exhibited some degree of toxicity at the higher doses examined, i.e., 160 or 640 mg/kg.

B. Antitrypanosomal Screening. Of the sixteen compounds submitted sufficient material was available to permit screening in this system for eight derivatives (5b,f,g,i,j, k,l,m,o). None of these compounds exhibited activity in the Rane/Ager test system (30).

VII. Appendices

A. Publications. Synthesis of 6-Benzyl- and 6-Phenethyl-2,4-Diamino-5,6,7,8-Tetrahydropyrimido|4,5-d| pyrimidines, T. J. Delia and S. M. Sami, J. Heterocyclic Chem. (in press).

Synthesis of 6-Aralkoxy-2,4-Diamino-5,6,7,8-Tetrahydropyrimido|4,5-d| pyrimidines and related derivatives, T. J. Delia, D. D. Kirt and S. M. Sami, manuscript in preparation.

B. Personnel. Thomas J. Delia, Principal Investigator
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TABLE I

5	% Yield	Solvent of Crystallization	M.P. Free Base	M.P. of HCL Salt	Mole Formula	Calculated Mole Weight	Analysis Calculated C	H	N	Found C	H	N
a . 3,4-Cl_2-C_6H_3-CH_2-	98	MeOH	194-196°	230-233°; d	$C_{13}H_{14}N_6Cl_2$	325.2	48.00	4.33	25.82	47.80	4.42	25.69
b 2,4-Cl_2-C_6H_3-CH_2-	92	$CHCl_3$ or DMSO-H_2O	155-157°	214-216°; d	$C_{13}H_{14}N_6Cl_2H_2O$	325.2	45.50	4.70	24.50	45.98	4.81	24.69
c 4-Cl_2-C_6H_4-CH_2-	97	EtOH	197-199°	240-241°; d	$C_{13}H_{15}N_6Cl\cdot 2HCl$	290.76	42.92	4.68	23.10	42.99	5.01	22.53
d 3-CF_3-C_6H_4-CH_2-	98	$CHCl_3$ or DMSO-H_2O	158-159°	213-215°; d	$C_{14}H_{15}N_6F_3\cdot 2HCL\cdot 2H_2O$	324.31	38.79	4.80	19.40	38.55	4.26	19.23
e 2-Cl-C_6H_4-CH_2-CH_2-	62.4	MeOH or MeOH-Et_2O	186-188°	212-217°	$C_{14}H_{17}N_6CL\cdot CH_3OH$	304.78	53.48	6.28	24.95	53.37	6.25	25.09
f 4-CL-C_6H_4-CH_2-CH_2-	76	MeOH	187-193°	212-217°; d	$C_{14}H_{17}N_6Cl\cdot CH_3OH$	304.78	53.48	6.28	24.95	53.82	6.31	25.07
g 3-Cl_2-C_6H_4-CH_2 CH_2-	96	MeOH	104-105.5°	206-210°; d	$C_{14}H_{17}N_6Cl\cdot CH_3OH$	304.78	53.48	6.28	24.95	53.33	6.21	25.28
h 3-CF_3-C_6H_4-CH_2CH_2-	80.6	MeOH or MeOH-H_2O	112-114°	--	$C_{15}H_{17}N_6F_3$	338.24	51.89	5.75	22.70	51.44	5.72	22.93
i 2,4-Cl_2-C_6H_3-CH_2CH_2-	80.2	$CHCl_3$ or DMSO-H_2O	186-188°	--	$C_{14}H_{16}N_6Cl_2$	339.23	49.75	4.74	24.69	49.73	4.77	24.72
j 2,6-Cl_2-C_6H_3-CH_2-CH_2-	76.1	MeOH-EtOH	200-202°	--	$C_{14}H_{16}N_6Cl_2$	339.23	49.75	4.74	24.69	49.57	4.75	24.73
k 2,6-Cl_2-C_6H_3-CH_2-O-	42	MeOH	230-231.5°	236.5-238°; d	$C_{13}H_{14}N_6Cl_2O$	341.2	45.75	4.11	24.63	45.64	4.14	24.58
l 2,4-Cl_2-C_6H_3-CH_2O-	43	EtOH	208-209.5°	239-241.5°; d	$C_{13}H_{14}N_6Cl_2O$	341.2	45.75	4.11	24.63	45.76	4.09	24.67
m 4-Cl_2-C_6H_4-CH_2-O-	56	MeOH	205-206.5°	251-253°; d	$C_{13}H_{15}N_6ClO$	306.76	50.90	4.89	27.40	51.02	5.08	27.41
n 3-CF_3-C_6H_4-CH_2-O-	37	$CHCl_3$-MeOH	200-202°	247-249° d	$C_{14}H_{15}N_6F_3O$	340.31	49.41	4.41	24.70	49.64	4.61	24.73
o 2,4-Cl_2-C_6H_3-O-CH_2-CH_2-O-	50	MeOH	180-182.5°	--	$C_{14}H_{16}N_6Cl_2O_2$	371.0	45.28	4.31	22.64	45.04	4.55	22.13
p 4-CL-C_6H_4-CH_2CH_2-O-	62	MeOH-H_2O	187-189°	--	$C_{14}H_{17}N_6ClO$	320.5	52.46	5.30	26.21	52.48	5.31	26.25

TABLE 2

SUMMARY OF BIOLOGICAL SCREENING RESULTS

Report Identification No.	Submitter Compound No.	W. R. Bottle No.	Screening Results Antimalarial	Antitrypanosomal
5a	SMS - 1	BH84175	-	
b	SMS - 5	BH84193	-	
		BH89063		-
c	SMS - 2	BH84184	-	
d	SMS - 12	BH84513	-	-
e	SMS - 20	BJ07100	-	-
f	SMS - 15	BJ07084	-	-
g	SMS - 18	BJ07093	-	-
h	SMS - 24	BJ09971	-	
i	SMS - 21	BJ07119	-	
j	SMS - 19	BJ01993	-	-
k	SMS - 6	BH86508	-	
		BH89072		-
l	SMS - 7	BH86517	-	
		BH89081		-
m	SMS - 8	BH86526	-	
		BH89090		-
n	SMS - 9	BH86535	-	
o	SMS - 23	BJ07128	-	-
p	SMS - 22	BJ09471		

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